described in Example 4. Chips were coated at the concentrations listed in the table below. The amount of immobilized biotinylated DNA was measured by reacting a pre-determined surface area with a sufficient volume of 1:1000 dilution of a streptavidin/horseradish peroxidase conjugate to cover the test surface. The conjugate and surface were incubated at room temperature for 10 minutes. The chips were rinsed 2 times with wash buffer and then water. The Kirkegaard and Perry two component TMB substrate was applied for 20 minutes at room temperature. A volume of the substrate was placed in a microtiter well with 50 ul of 2.5 N sulfuric acid and 50 ul of water. The absorbance at 450 nm was measured in a microtiter plate reader. The minimum detectable amount of biotinylated DNA was correlated to the copy number of capture probe used in the initial coating solution. The results are shown below:

REMARKS

This reply is responsive to a Notice of Non-Compliant Amendment mailed on July 24, 2002. This amendment provides a clean version of complete replacement paragraphs to the specification, submitted previously on February 11, 2002, as well as a marked-up version of the complete replacement paragraphs to the specification previously submitted.

The application has now been amended to refer to updated sequence identifiers. The replacement paragraphs incorporate the updated sequence identifiers and the marked-up pages show where the sequence identifiers were added. The sequence listing and the CRF were submitted with the Notice To File Missing Parts on February 11, 2002.

The Notice of Non-Compliant Amendment mailed on July 24, 2002 states that the amendment submitted on February 11, 2002 does not include a clean version of the amended claims and a mark-up version of the claims. However, the claims were not amended in the amendment submitted on February 11, 2002. A complete, new specification was submitted on February 11, 2002 but the claims remained the same as they were filed on October 18, 2001.

The Examiner is invited to contact the undersigned if such communication would expedite the prosecution of the application.

Respectfully submitted,

Date August 21, 2002 By _____

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Michael A. Whittaker
Attorney for Applicant
Registration No.

Version with markings to show changes made

IN THE SPECIFICATION:

Please replace the paragraph starting on Line 21 on page 39 with the following amended paragraph:

DNA capture probe was coated onto these wafer surface from a solution containing 50 mM sodium citrate, pH 6.0, 0.1 mg/ml carrier DNA, sheared herring sperm DNA, and 600 μM biotinylated DNA, 26-mer. The probe sequence was 5'CGCTAATATCAGAGAGATAACCCAC-3' (SEQ. ID NO.1). Wafers were incubated in this solution overnight at 4°C. Wafers were removed from the solution and washed with 1x phosphate buffered saline containing 0.2% Tween 20™ detergent (PBS/Tween). The wafers were coated in a BSA (bovine serum albumin) solution for 3 hours at 65°C. There wafers were then rinsed with PBS/Tween detergent.

Please replace the paragraph starting on Line 19 on page 40 with the following amended paragraph.

Wafers coated with T-polymer (see Example 1) were coated for 56 hours at 4°C in a solution containing 50 mM sodium citrate buffer, pH 6.0, 5X SSC, and 20 μg/ml of the ssDNA capture probe complimentary to M13mp18. The probe sequence was CGCTAATATCAGAGAGAT AACCCAC (SEQ. ID NO. 1). Probe coated wafers were removed from coating solution and placed into a blocking solution containing 5X Denhart's solution, 0.5% SDS, 1mg/ml carrier DNA, and 25mM buffer at pH 6.5. They were incubated 16-18 hours at 4°C and then rinsed with phosphate buffered saline containing 0.0005% TWEEN20 detergent at pH 7.4. Capture probe coated wafers were hybridized with M13mp18 plasmid overnight at 60°C in a solution containing 1X Denhardt's solution, 0.5% SDS, 25 mM MES, pH 6.5, 0.2 mg/ml carrier DNA, 5X SSC, a final concentration of M13mp18 was 500ng/ml, 1 ng/ml or 100pg/ml. The final hybridization step occurred under the same solution and incubation conditions as the previous step with a final biotinylated amplifying probe concentration of 92μM. The amplifying probe contains strand sequence from 6249 to 6273 and was biotinylated at

residue 6261. The sequence is GCAGGTCGACTGTAGCAGGATGCCGG (SEQ. ID NO. 2). All appropriate controls were performed. Wafers were incubated with a steptravidin alkaline phosphatase conjugate. Precipitating substrate, BCIP/nitroblue tetrazolium, was used to generate an increase in thickness at the surface of the wafer. Thickness increases were measured using an absolute ellipsometer (Gaertner). Results for the experiment are shown in Figure 2. From this experiment, it was concluded that a sensitivity of 1 ng/ml and potentially as low as 100 pg/ml was achieved. This translates to a copy number of roughly **10 10** for a very un-optimized assay.

Please replace the paragraph starting on Line 29 on page 43 with the following amended paragraph.

An 18 mer DNA/RNA chimera was utilized as the capture probe. The probe was biotinylated at the 5' end and has a ribonucleotide cytosine on the 3' end. The DNA sequence was 5'-CGAAGCTTGGATCCGCC-3' (ribose) (SEQ. ID No. 3). The covalently attached capture probe was treated with S1 nuclease to degrade the entire probe from the surface. The S1 nuclease was mixed in a solution of 0.2 mM NaCl, 0.05 M sodium acetate pH 5.4, 1 mM ZnSO, and 0.5% glycerol to a final concentration of 2 units/ml. A section of the wafer was submerged into 7 ml of the enzyme solution and incubated for 10 minutes at 37°C. Wafers were rinsed prior to enzyme treatment in water for 2 hours at 45°C. The enzyme solution was decanted into test tubes and a small volume of water used to rinse the wafers. The combined solution was dried with a SpeedVac system. The pellet was extracted into acetone and the solution dried. These pellets were re-suspended in 70 μl of water and the A_{260} measured in a microcuvette. The surface density of the probe was determined to be 50 ng/**cm^2**. Control surfaces where no $NaIO_4$ was used, no covalent attachment, did not generate signal. Control surfaces without DNA or without S1 nuclease gave no signal.

Please replace the paragraph starting on Line 30 on page 44 with the following amended paragraph:

An 8-mer was synthesized and was 5' biotinylated and had a 3' ribose. The DNA sequence was 5'AAAGATGTA (ribose)-3' (SEQ ID NO. 4). The 8-mer was immobilized using the 15:1 periodate:probe ratio to a TC7 coated optical substrate as described in Example 4. Chips were coated at the concentrations listed in the table below. The amount of immobilized biotinylated DNA was measured by reacting a pre-determined surface area with a sufficient volume of 1:1000 dilution of a streptavidin/horseradish peroxidase conjugate to cover the test surface. The conjugate and surface were incubated at room temperature for 10 minutes. The chips were rinsed 2 times with wash buffer and then water. The Kirkegaard and Perry two component TMB substrate was applied for 20 minutes at room temperature. A volume of the substrate was placed in a microtiter well with 50 ul of 2.5 N sulfuric acid and 50 ul of water. The absorbance at 450 nm was measured in a microtiter plate reader. The minimum detectable amount of biotinylated DNA was correlated to the copy number of capture probe used in the initial coating solution. The results are shown below: